sarcomere lengths might be less uniform after active stretch; however, further testing will increase the sample size to 20. This will allow for a more general idea of the development of sarcomere length non-uniformities following active stretch and might provide additional insight into the mechanism of RFE.

3863-Pos Board B591
Does Calcium Sensitivity Increase after Active Stretch in Skinned Muscle Fibres?
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Introduction Isometric force after active stretch of muscles is higher than the purely isometric force at the corresponding lengths. This property is termed residual force enhancement (RFE). Active force in skeletal muscle depends on calcium attachment characteristics to troponin. Passive force has been shown to influence calcium attachment characteristics in cardiac muscle, specifically the sarcomere length-dependence of Ca-sensitivity. Since one of the mechanisms proposed to explain RFE has been the increase in passive force that results from the engagement of titin upon activation and stretch, our aim was to test whether Ca-sensitivity was changed after active stretch and whether this change was related to titin.
Methods Tension-pCa curves were established in three groups of fibres for reference and active stretch contractions at a sarcomere length of 3.0µm. Group 1 (n=13) was formed of skinned fibres without any chemical treatment. Fibres in Group 2 (n=12) were treated with trypsin before establishing the tension-pCa curves. In Group 3, fibres (n=9) were osmotically compressed by dextran T-500 after treatment with trypsin.
Results and discussion After active stretch, Ca-sensitivity was increased in Group 1 fibres. When titin was eliminated using trypsin, this increased Ca-sensitivity was abolished. This means that titin is probably responsible for the increase in Ca-sensitivity. The mechanism by which titin may modulate Ca-sensitivity is by reducing the myofilament lattice spacing by generating a radial force that compresses the myofilaments. However, when, in the absence of titin, fibres were compressed, they did not show any difference in Ca-sensitivity between reference and active stretch contractions. These results suggest that Ca-sensitivity increases after active stretch compared to isometric contractions and that titin must be present in order for this increase to occur.

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Thixotropy of Muscle Fibers Probed with Sinusoidal Oscillations
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Mechanical properties of individual muscle fibers from rabbit psoas were probed by applying sinusoidal length changes at one end of the fiber and measuring the resulting force at the other end. At frequencies above 1 Hz, this oscillation resulted in a history-dependent reduction in muscle fiber stiffness in activated muscle fibers. Such a reduction in muscle stiffness in response to force is referred to as thixotropy. Perturbing the actin-myosin interaction by treatment with EDTA and blebbistatin caused this effect to disappear, suggesting that this thixotropy results from cross-bridge interactions. At frequencies less than 1 Hz, oscillation resulted in a history-dependent increase in muscle fiber stiffness in activated fibers. Disruption of actin-myosin interactions has no effect on this negative thixotropy, suggesting a different mechanism is at play.

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HOP Skip and Jump; but How?
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Apart from fast, efficient and coordinated contraction of several skeletal muscles, running and jumping require effective absorption of the kinetic energy of the body during the landing phase, to absorb impact forces and prevent injury. This is done not only by joints, bones and tendons, but also by activated muscles which resist stretch. During lengthening, skeletal muscle bears higher force and has higher instantaneous stiffness than during isometric contraction, and yet consumes very little ATP. These properties allow muscle to absorb the energy of a fall or landing by stretching. Our work shows how the acto-myosin molecules change their structure and interaction to implement these physiologically useful mechanical and thermodynamical properties. The low angle x-ray diffraction pattern of rabbit skeletal muscle fibers was monitored during isometric contraction and compared to that during ramp stretch. The experiments were carried out at physiological temperature, using low-angle X-ray synchrotron radiation at ID2, ESRF, Grenoble. The intensities of the off-meridional layer lines and fine interference structure of the meridional M3 myosin X-ray reflection were resolved. Mechanical and structural data show that upon stretch the fraction of actin-bound myosin heads is higher than during isometric contraction. This finding accounts for the higher stiffness and greater energy absorbing capacity of stretched muscle compared to isometric. However the intensities of the actin layer lines are lower than during isometric contraction. These results suggest that during stretch, a significant fraction of actin-bound heads is bound weakly or non-stereo-specifically. That is, the actin-bound myosin heads are disordered azimuthally but are stiff axially. As the strong or stereo-specific myosin binding to actin is necessary for actin activation of the myosin ATPase, this finding explains the low metabolic cost of energy absorption by muscle during the landing phase of locomotion.

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Active and Passive Failure of Permeabilized Muscle Fibres from the Rabbit Psoas
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It has been shown that muscle failure, defined as a tear in the muscle, differs depending on the state of activation of the muscle. Active muscles tear at higher forces than passive muscles; however, irreversible damage to muscles occurs prior to the appearance of a tear. This leads us to define failure instead as a drop in force during stretching. Leonard *et al.* (2010) showed that, in myofibrils from the rabbit psoas, active specimens failed at much higher forces than passive ones and that both failed at lengths well beyond myofilament overlap. The purpose of this study was to investigate failure as defined by Leonard *et al.* in the same muscle, but in isolated permeabilized fibres instead of myofibrils.
Permeabilized fibres were prepared as described in Joumaa *et al.* (2013). Once mounted between a force transducer and a length controlling motor, fibres were positioned at optimal sarcomere length (determined with laser diffraction) and the corresponding fibre length (L_0) was measured. Fibres that were failed actively were maximally activated to allow full tension to develop and then stretched at approximately 25% L_0/s. Passively-failed fibres were maximally activated, allowed to relax, and then stretched in the same manner as active fibres. Failure was defined as the appearance of a negative slope on the force-time history.
Both passively- and actively-stretched fibres failed at lengths beyond myofilament overlap (active = 5.82 µm; passive = 5.58 µm). Active fibres failed at relatively higher forces than passive fibres (active = 193%F_0; passive = 157%F_0). Although the differences between active and passive fibres were not as pronounced as that observed in Leonard *et al.*'s myofibrils, a similar relationship persisted; that is, active fibres failed at higher forces than passive fibres despite being beyond myofilament overlap.

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Membrane-Sealant Copolymers Confer Protection to Dystrophic Skeletal Muscle in Vitro and in Vivo
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Muscle membrane vulnerability is a hallmark of Duchenne Muscular Dystrophy (DMD), an X-linked disease that results in progressive skeletal muscle weakness and cardiomyopathy. Cardiac disease is an increasing cause of death in DMD and there is currently no cure for DMD. One unique therapeutic approach is the use of membrane sealants to protect the fragile dystrophic muscle membranes from mechanical stress. We propose a structure-function strategy in understanding the mechanism by which block copolymers may protect dystrophic cardiac and skeletal muscles. Poloxamer 188 (P188) is a membrane sealant that has been shown by us and others to protect the fragile dystrophic myocardium under physiological stress but appears to have